P513 A Rapid Approach for Diagnosing *Ceriodaphnia dubia* Reproductive Failure

Lazorchak, J.M.[1], Smith, M.E.[2], Tucker, M.W.[3], Andrews, P.L.3, Bennett, M.C.[2], Haring, H.J.[2], and H.J. Allen*[4]

[1].USEPA, NERL, MERB, [2].SoBran Inc., c\o USEPA, [3].USEPA Region 7, [4].USEPA, NRMRL, WQMB, *Presenter

Abstract

Sources of failure in *C. dubia* culture and testing can be difficult to determine without outside assistance. Investigation of these failures can be time consuming. In the current study, USEPA Region 7 laboratory suffered a drop in reproduction in the *C. dubia* cultures, with weekly averages less than the required 15 young/female minimum. Repeated attempts to restart the cultures with animals from an outside source failed. USEPA-NERL Cincinnati, OH agreed to participate in an inter-lab study to determine the cause of the culture failure. Preliminary analysis of the culture failure pointed to a problem related to the food or water being used by Region 7. Specifically, the cereal leaves Region 7 used to prepare Yeast/Cereal Leaves/Trout Chow (YCT) were outdated. Region 7 supplied NERL with culture water, YCT and algae. NERL supplied Region 7 with culture water, YCT and algae, along with animals from a successful culture. Eight experimental combinations of food and water were developed, such that the performance of each individual component could be determined. After a 7-day culture period, it was determined that the culture failure in Region 7 was due to the YCT. *C. dubia* reproduction in the 8 cultures (4 in each laboratory) that used Region 7 YCT ranged from 4.2 young/female to 17.8 young/female. Reproduction in the 8 cultures that used NERL YCT ranged from 17.4 young/female to 30.9 young/female. Region 7 replaced cereal leaves with fresh alfalfa and digested trout chow with digested flake food in the preparation of YCT. Subsequent success of the Region 7 cultures (weekly averages in excess of 20 young/female) confirmed that the problem was resolved. Systematic round-robin testing is an effective tool that can rapidly diagnose and resolve problems related to *C. dubia* culturing.

Introduction

Culture of the freshwater cladoceran *Ceriodaphnia dubia* is necessary for conducting aquatic toxicity tests required by USEPA. Maintenance of these cultures requires skill and resolving culture problems can be difficult. In this study, USEPA Region 7 experienced a problem with the *C. dubia* cultures they maintain, with reproduction dropping below acceptable levels (less than 15 young/female). The Regional Biologist contacted the USEPA-NERL laboratory in Cincinnati (EPA-Cinn), to request assistance in resolving the problem. This study was developed as an attempt to diagnose the cause(s) of the poor culture reproduction experienced by Region 7. Region 7 and EPA-Cinn exchanged food and water, then conducted concurrent culture tests using *C. dubia* supplied by EPA-Cinn. The goal was to isolate the various food and water components used to culture the animals, thereby determining which food or water component was causing the Region 7 culture problems.

Problem Diagnosis

- Isolate all variables, in this case food and water.
- Develop a matrix of all possible food and water combinations, see Tables 1 and 2.
- Locate source of quality animals, in this case EPA-Cinn.
- For an inter-laboratory study, exchange food and water, so that each lab could use an aliquot of the same food and/or water being used by the other participant.

Table 1. Water and Food Regimes-Region 7 water

1		3	
Region 7 water		Region 7 water	
Region 7 YCT		Cincinnati YCT	
Region 7 algae		Region 7 algae	
2		4	
Region 7 water		Region 7 water	
Region 7 YCT		Cincinnati YCT	
Cincinnati Algae		Cincinnati algae	

Table 2. Water and Food Regimes-EPA-Cinn water

5		7	
EPA-Cinn water		EPA-Cinn water	
Region 7 YCT		Cincinnati YCT	
Region 7 algae		Region 7 algae	
6		8	
EPA-Cinn water		EPA-Cinn water	
Region 7 YCT		Cincinnati YCT	
Cincinnati algae		Cincinnati algae	

USEPA Region 7

USEPA Cincinnati

Culture Method Summary

- 25°C temperature
- 1 neonate/replicate
- 10 replicates
- 15 ml/replicate
- fed 0.1 ml YCT and 0.1 ml algae daily
- water renewed, young counted daily

Food Materials and Preparation

- Tropical fish flakes used in place of trout chow.
- Alfalfa used in place of cereal leaves.
- 5 grams of flakes blended in 1L deionized water, aerated 7 days to ferment.
- 5 grams alfalfa blended in 1L deionized water.
- 5 grams yeast blended in 1L deionized water.
- Equal portions of each combined to make yeast, cereal leaves, trout chow (YCT) food. All were filtered through 60 micron screen before mixing.

Culture Water

- Each laboratory used moderately hard reconstituted water (MHRW), supplemented with 1 ug/l selenium.
- Each prepared MHRW prior to the start of the study, allowed to age at least 3 days.
- Each lab provided 8L of MHRW for use by the other participant in the culture study.

Chart 1. Graph of #young/female and Coefficient of variance for Region 7 and all water/food regimes.

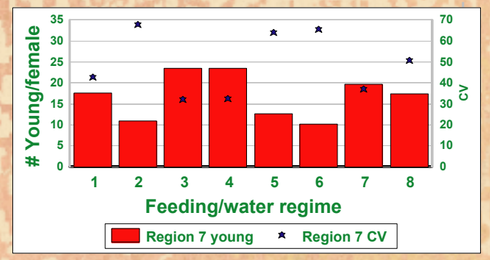
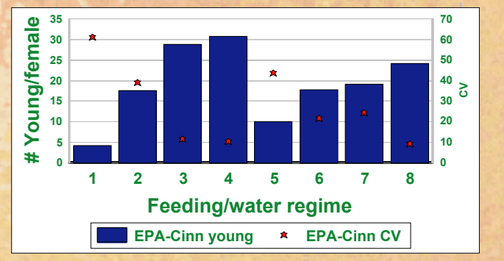


Chart 2. Graph of #young/female and Coefficient of variance for EPA-Cinn and all water/food regimes.



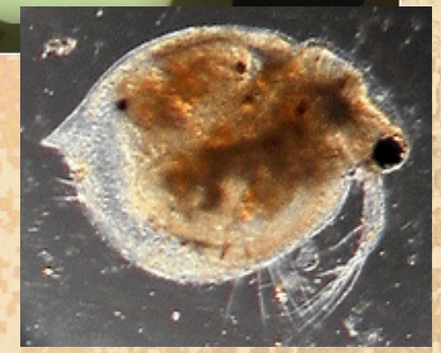
Results from Region 7 Chart 1

- Use of YCT from EPA-Cinn improved culture performance.
- Young/female averaged 17.5 in treatment 1, all Region 7 food and water.
- Young/female averaged 23.4 in treatment 4, Region 7 food and EPA-Cinn water.
- Young/female averaged 12.7 in treatment 5, Region 7 food and EPA-Cinn water.
- Young/female averaged 17.4 in treatment 8, EPA-Cinn food and water.

Results from EPA-Cinn Chart 2

- Animal reproduction was better in treatments using food from EPA-Cinn.
- Young/female averaged 4.2 in treatment 1, all Region 7 food and water.
- Young/female averaged 30.9 in treatment 4, Region 7 water and EPA-Cinn food.
- Young/female averaged 10.1 in treatment 5, Region 7 food and EPA-Cinn water.
- Young/female averaged 24.3 in treatment 8, EPA-Cinn food and water.

Counting Live and Young in a Ceriodaphnia dubia Test



Ceriodaphnia dubia

Discussion

- Data indicates the poor culture performance Region 7 experienced was due to a problem related to YCT.
- Use of YCT from EPA-Cinn resulted in higher average young production.
- In 2 of the Region 7 culture test treatments (3 and 7, Chart 2) use of Region 7 algae with EPA-Cinn YCT resulted in animal reproduction greater than or equal to that found with EPA-Cinn food only.

Conclusion

This study indicates the cause of the Region 7 culture failure was diagnosed in a one week culture cycle.
Isolating all variables allowed each to be evaluated independently, simplifying diagnosis .
Use of quality animals from an outside source provides data that are based on optimal animal reproduction.
Performing this type of study using animals of marginal quality could produce suspect data.

Prepared by CSC Inc. for ORD/NERL/Cincinnati, OH - November 2002